culture in the presence and absence of serum (DMEM media). With patient ND, additional samples from, surrounding sub-dura, and dura were also collected and treated as described above. Also, control skin fibroblast cultures from patient BD were obtained on the same day as tumour resection, and treated in the same way as the tumour samples. Samples from patient BD were labeled as follows: 1: tumour conditioned media (TCM-BD); 2: skin conditioned media (SCM-BD). Samples from patient ND were labeled as follows: 1: Tumour conditioned media (TCM-ND); 2: sub-dura conditioned media (SDCM-ND); 3: dura conditioned media (DCM-ND); 4: fluid surrounding intracranial-tumour (FST-ND). All samples were collected from culture cycles in which cells were grown in serum-free DMEM media, unless indicated in the text by addition of 'serum supplemented' to the above abbreviations.

Concanavilin A affinity chromatography of TCM:

Concanavilin-A affinity chromatography of tumour conditioned medium (TCM) from patient ND, performed in accordance with Example 1 resulted in the isolation of high and low affinity fractions (HCA, and LCA respectively). Both HCA and LCA fractions were eluted with α-methyl-D-glucopyranoside (0.5M) elution buffer. Briefly, partial purification of TCM proteins was carried out by Conacanavilin A affinity chromatography using a method described by (Wagner, Gen. Comp. Endocrinol. 63 (1986), 481-491), with modifications. Concanavilin A Sepharose (Pharmacia Code No: 17-0440-01, 14 ml), in 20% Ethanol, was first washed with several column volumes of water, and then equilibrated in running buffer (CRB; 0.06M Sodium phosphate pH 7.2 and 0.5M NaCl). The equilibrated slurry was then added to a 12 mm X 115 mm Pharmacia screw top column, and three column volumes of CRB running buffer added at a flow rate of 0.4 ml/min (FPLC/HPLC millenium Waters chromatography system). Conditioned media equilibrated in CRB buffer (10 ml), was then added to the column and allowed to bind. The column was then washed with several column volumes of CRB loading buffer, and elutions of bound proteins was then carried out by addition of sodium phosphate elution buffer (ERB; 60 mM pH 7.2/ 0.5M NaCl/0.5M α-methyl-D-glucopyranoside/0.01% azide), at a flow rate of 0.2 ml/min (40 ml). High affinity proteins were eluted after incubation of the column overnight in ERB buffer

followed by a second passage of ERB buffer at 0.2 ml/min. Elution profiles for both high and low concanavilin A-affinity TCM-proteins were identical and produced a single symmetrical peak at ~1.6 column volumes. Peak LCA represented 1/3 the total mass of peak HCA, and 1 ug of HCA material was retrieved from 10 ml of tumour conditioned media (TCM), from patient ND.

SDS-PAGE of TCM and concanavilin A fractions:

Tumour conditioned medium, conditioned media and concanavilin A peaks (HCA and LCA), were separated by SDS-PAGE and visualized after Sybr-Orange staining. SDS-polyacrylamide gel electrophoresis was carried out using a Novex NuPAGE™ Electrophoresis system consisting of 4-12% Bis-Tris acrylamide-gradient gels (pH 6.4), and MOPS-SDS (50 mM 3-[N-morpholino] propane sulfonic acid; 50 mM Tris-base; 3.5 mM SDS; 1.0 mM EDTA; pH 7.7) running buffer. Runs were carried out at a constant voltage of 200 for 50 min. Samples were denatured at 70°C for 10 minutes in NuPage LDS sample buffer (10% glycerol; 1.7% Tris-Base; 1.7% Tris-HCl; 2% Lithium Dodecyl Sulfate; dithiothreitol 50 mM; 0.015% EDTA; 0.075% Serva Blue G250; 0.025% Phenol red; pH7.5 final concentration). NuPage antioxidant was added to the upper electrophoresis chamber as recommended by the manufacturers. Following electrophoresis proteins were stained by incubating the gels in 7.5% acetic acid supplemented with SYPRO-Orange. Visualization of proteins was achieved after UV illumination using a Bio-Rad FluorImager gel-imaging system. HCA and LCA fractions stained positive for two proteins at 56 kDa and 200 kDa respectively and gave identical profiles. Conditioned media (patient ND), from intracranial-tumour, sub-dura (immediately adjacent to tumour in the patient), and dura material contained several major bands spanning ~50-80 kDa. A prominent band was present in all preparations at ~ 66 kDa with a weaker very high molecular weight component at ~ 200 kDa present in tumour and sub-dura. The relative intensity of the ~ 200 kDa was highest in the tumour material, and absent in the dura. A diffuse set of bands at ~ 55-60 kDa was present in tumour and sub-dura but absent in the dura conditioned media (patient ND). Conditioned media from skin and media control did not reveal any staining for protein. Conditioned media from patient BD and EM gave similar profiles except for the absence of the high molecular weight protein at 200 kDa.

Non phosphaturic tumour tissues from patients LA and SL, and also skin controls all contained the 66 kDa band and also diffuse staining at 50-60 kDa. Concanavilin-A affinity peaks HCA and LCA were enriched for the high molecular weight 200 kDa band and also contained proteins from the 50-66 kDa range. Conditioned media from bone cell lines HTB96 and SaOs2 gave almost identical protein profiles to tumour conditioned media from OHO-patient ND. The 200 kDa band intensity in SaOS2 was reduced relative to TCM from brain tumour (patient ND), sub-dura (patient ND), and CM from HTB96.

Immuno-blotting and glycoprotein staining of TCM and purified fractions:

For western-blotting, proteins were transferred to PVDF membranes (Amersham), using submarine electrophoresis. After SDS-PAGE electrophoresis, gels were equilibrated in transfer buffer: 25 mM Tris-HCl; 0.38 M glycine; 0.2% SDS (TB) for 1 h at room temperature. PVDF membranes were cut to size, briefly rinsed in methanol, washed in distilled water, and then equilibrated in TB. The equilibrated gel and PVDF membrane were then sandwiched between filters and placed in a cassette. The cassette was then placed in a Hoeffer system submarine electroblotter with TB buffer and cooling maintained at 4°C by thermocooler. Transfer of proteins was then carried out by positioning the PVDF end of the sandwich towards the anode, and electrophoresis at a constant 0.4 A (45V), for 45 min. Blots were screened with 1/1000 dilution of pre-Anti-op antisera, post-Anti-op-antisera, or calmodulin conjugated to alkaline phosphatase using the methods described in the Enhanced-Chemiluminescence kit (Amersham; ECL+), or the calmodulin affinity detection kit (Stratagene) respectively. Chemiluminescence, was detected and filmed using the Bio-Rad FluorImaging system, and the calmodulin-affinity binding was visualized using the colourometric system discussed earlier for clone detection (Stratagene). Biotinylated molecular weight markers (Amersham), were used as internal controls to asses transfer and molecular weight. Streptavidin conjugated to horse radish peroxidase (HRP), was added to the secondary antibody (goat-anti-rabbit IgG conjugated to HRP), to facilitate visualization of the biotinylated-markers via chemiluminescence.

Western blots of phosphaturic tumour-conditioned-media (TCM), from OHO-patients gave positive chemiluminescent bands when screened with pre-absorbed